

CLAIMS

1. A purified polynucleotide having a nucleic acid sequence selected from the group consisting of SEQ ID Nos. 10, 12, 14, 16, 18, 20 and 21.
2. An expression vector comprising the polynucleotide of claim 1.
- 5 3. A host cell transformed with the expression vector of claim 2.
4. An isolated polypeptide encoded by the expression vector of claim 2.
5. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos. 1-3, 5, 7-9, 11, 13, 15, 17, 19 and 22.
6. An antibody which binds to the polypeptide of claim 4.
7. An antibody which binds to the polypeptide of claim 5.
8. A composition comprising Smad1 and an interaction partner protein selected from the group consisting of HsN3, antizyme, PAG, GST, tumor associated gene, AIP4, U1SnRNP, TRIP4, Ran GTP binding protein 5, P0 acidic ribosomal phosphoprotein, β-tubulin, KIAA 00104, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 7, SEQ ID No. 11, SEQ ID 15 No. 13, SEQ ID No. 15, and SEQ ID No. 19.
9. A composition comprising Smad2 and an interaction partner protein selected from the group consisting of GST, AIP4, TRIP4, KIAA 00104 and SEQ ID No. 3.
10. A composition comprising Smad3 and an interaction partner protein selected from the group consisting of HsN3, KIAA 00104, HEF1, FKBP25, AIP4, SnRNP C, RBP2, TRIP4,

hnRNP A1, GST, SEQ ID No. 11, SEQ ID No. 15, SEQ ID No. 13, SEQ ID No. 4, SEQ ID No. 17 and SEQ ID No. 22.

11. A screening method to identify a compound which modulates the interaction of a first protein with a known interaction partner of said first protein, said method comprising the
5 steps of:

a) contacting a protein comprising said first protein with a protein comprising said interaction partner in both the presence and absence of said compound; and

b) detecting the amount of said interaction partner bound to said first protein, wherein an increase in bound interaction partner in the presence of said compound indicates said compound is an agonist of said interaction, and a decrease in bound interaction partner in the presence of said compound indicates said compound is an antagonist of said interaction.
10

12. The method of claim 11, wherein said first protein is Smad1 and said known interaction partner is selected from the group consisting of HsN3, antizyme, PAG, GST, tumor associated gene, AIP4, U1SnRNP, TRIP4, Ran GTP binding protein5, P0 acidic ribosomal phosphoprotein, β-tubulin, KIAA 00104, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 7, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 15, and SEQ ID No. 19.
15

13. The method of claim 11, wherein said first protein is Smad2 and said interaction partner is selected from the group consisting of GST, AIP4, TRIP4, KIAA 00104 and SEQ ID No. 3.
20

14. The method of claim 11, wherein said first protein is Smad3 and said interaction partner protein is selected from the group consisting of HsN3, KIAA 00104, HEF1, FKBP25,

AIP4, SnRNP C, RBP2, TRIP4, hnRNP A1, GST, SEQ ID No. 11, SEQ ID No. 15, SEQ ID No. 13, SEQ ID No. 4, SEQ ID No. 17 and SEQ ID No. 22.

15. The method of claim 11, wherein said first protein is HsN3 and said interaction partner protein is selected from the group consisting of antizyme, GST, PAG, FKBP25, TRIP4, 5 HEF1, AIP4, SnRNP C, hnRNP A1, TGF- β type II receptors, BMP type I receptor ALK3, FNTA, GGTB, KIAA 00104, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 7, SEQ ID No. 11, SEQ ID No. 13 and SEQ ID No. 22.

16. The method of claim 11, wherein said first protein is antizyme and said interaction partner protein is selected from the group consisting of enolase, PAG, tumor associated gene, hnRNP A1, TRIP4, AIP4, HEF1, SnRNP C, KIAA 00104, HnRNPA1, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 7, SEQ ID No. 11 and SEQ ID No. 22.

17. The method of claim 11, wherein said first protein is SEQ ID No. 3 and said interaction partner protein is selected from the group consisting of HsN3, antizyme, SEQ ID No. 7, AIP4, the cytoplasmic domain of ALK2 and the cytoplasmic domain of ALK5.

15 18. The method of claim 11 performed in vitro.

19. The method of claim 11 performed in yeast.

20. The method of claim 11 performed in mammalian cells.

21. A method to identify a candidate compound which modulates the interaction between a first protein and a known interaction partner protein of said first protein in yeast cells comprising the steps of:

- DRAFT
PATENT
APPLICATION
- 5 a) transforming yeast cells with expression constructs comprising:
- i) a reporter gene functionally linked to a DNA sequence bound by a second protein;
- ii) a gene comprising a first protein fused to a DNA binding domain of said second protein; and
- iii) a gene comprising said known interaction partner protein of said first protein and a transactivation domain;
- 10 b) culturing the transformed yeast cells of a) in the presence and absence of said candidate compound; and
- c) detecting expression of said reporter gene, wherein an increase in reporter gene expression in the presence of said candidate compound indicates said candidate compound is an agonist of said interaction, and a decrease in reporter gene expression in the presence of said candidate compound indicates said candidate modulator is an antagonist of said interaction.

22. A method to identify a candidate compound which modulates the interaction
15 between a first protein and a known interaction partner protein of said first protein in mammalian cells comprising the steps of:

- 20 a) transfecting a mammalian cell line with expression constructs comprising:
- i) a reporter gene functionally linked to a DNA sequence bound by a second protein;
- ii) a gene comprising a first protein fused to a DNA binding domain of said second protein; and

- 10
11
12
13
14
15
16
17
18
19
20
- iii) a gene comprising said known interaction partner protein of said first protein and a transactivation domain;
- b) culturing the transfected mammalian cell line of a) in the presence and absence of said candidate compound; and
- 5 c) detecting expression of said reporter gene, wherein an increase in reporter gene expression in the presence of said candidate compound indicates said candidate compound is an agonist of said interaction, and a decrease in reporter gene expression in the presence of said candidate compound indicates said candidate compound is an antagonist of said interaction.
23. The method of claim 22 wherein said first protein and said known interaction partner protein of said first protein participate in cell signaling by TGF- β family member ligands.
24. The method of claim 22 wherein said first protein is a Smad and said known interaction partner of said first protein is a Smad interacting protein.
25. A method to identify a candidate compound which modulates the activity of an enzyme comprising the steps of:
- a) expressing said enzyme from a recombinant expression construct; and
- b) measuring the activity of said enzyme in the presence and absence of said candidate compound, wherein an increase in the activity of said enzyme in the presence of said candidate compound indicates said candidate compound is an agonist of the activity of said enzyme, and a decrease in the activity of said enzyme in the presence of said candidate compound indicates said candidate compound is an antagonist of the activity of said enzyme.

26. The method of claim 25 wherein said enzyme is expressed in a cell free system.

27. The method of claim 25 wherein said enzyme is expressed in cultured cells.

28. The method of claim 25 wherein said enzyme is GST.
29. The method of claim 25 wherein said enzyme is a phosphatase.
30. The method of claim 25 wherein said enzyme participates in cell signaling by TGF- β family member ligands.

5 31. A method for monitoring the proteasome-mediated proteolysis of a protein comprising the steps of:

- a) contacting an isolated polypeptide comprising a protein of interest with isolated proteasomes and a mammalian cell extract in the presence and absence of a specific proteasome inhibitor; and
- 10 b) detecting the amount of said protein of interest, wherein a decrease in said protein of interest occurring in the absence, but not the presence of said proteasome inhibitor indicates proteasome-mediated degradation of said protein.

15 32. A method to identify a candidate compound which modulates the proteolysis of a protein comprising the steps of:

- 15 a) transforming yeast cells with expression constructs comprising:
- i) a hybrid protein comprising from the amino terminus to the carboxyl terminus a DNA binding domain, a protein of interest and a transactivation domain; and
- ii) a reporter gene comprising a DNA sequence bound by said DNA binding domain and transactivated by said transactivation domain;
- 20 b) culturing said cells in the presence and absence of said candidate compound; and

10-2660
c) detecting the amount of reporter gene expression, wherein a decrease in reporter gene expression in the presence of said candidate compound indicates said candidate compound is an agonist of the proteolysis of said protein of interest, and an increase in reporter gene expression in the presence of said candidate compound indicates said candidate compound is an
5 antagonist of the proteolysis of said protein of interest.

33. A method to identify a candidate compound which modulates the proteolysis of a protein comprising the steps of:

- a) transforming yeast cells with expression constructs comprising:
- i) a hybrid protein comprising from the amino terminus to the carboxyl terminus a DNA binding domain, a protein of interest and a transactivation domain;
 - ii) a reporter gene comprising a DNA sequence bound by said DNA binding domain and transactivated by said transactivation domain; and
 - iii) a constitutively active mutant of a TGF- β family ligand type I receptor;
- and

15 b) culturing the yeast cells of a) in the presence and absence of said candidate compound; and

c) detecting the amount of reporter gene expression, wherein an increase in reporter gene expression indicates said candidate compound is an antagonist of proteolysis of said protein of interest, and a decrease in reporter gene expression indicates said candidate compound is an
20 agonist of the proteolysis of said protein of interest.

34. The method of claim 32 wherein said protein of interest participates in cell signaling by TGF- β family member ligands.

35. The method of claim 33 wherein said protein of interest participates in cell signaling by TGF- β family member ligands.

36. A method for monitoring the proteolysis of a protein of interest in mammalian cells comprising the steps of:

- 5 a) transfecting a mammalian cell line with expression constructs comprising:
 i) a hybrid protein comprising from the amino terminus to the carboxyl terminus a DNA binding domain, a protein of interest and a transactivation domain;
 ii) a reporter gene, comprising a DNA sequence bound by said DNA binding domain, and transactivated by said transactivation domain; and
10 b) detecting the expression of said reporter gene, wherein expression of said reporter gene indicates that said protein of interest is intact.

37. A method to identify a candidate compound which modulates the proteolysis of a protein of interest in mammalian cells comprising the steps of:

- 15 a) transfecting a mammalian cell line with expression constructs comprising:
 i) a hybrid protein comprising from the amino terminus to the carboxyl terminus a DNA binding domain, a protein of interest and a transactivation domain;
 ii) a reporter gene, comprising a DNA sequence bound by said DNA binding domain, and transactivated by said transactivation domain;
 b) culturing said cell line in the presence and absence of said candidate compound;
20 and

10
c) detecting the amount of reporter gene expression, wherein an increase in said reporter gene expression in the presence of said candidate compound indicates said candidate compound is an antagonist of proteolysis, and a decrease in said reporter gene expression in the presence of said candidate compound indicates said candidate compound is an agonist of
5 proteolysis.

15 38. A method to identify novel, tissue-specific Smad interactors comprising the steps of:

- a) transforming yeast cells with expression constructs comprising:
10 i) a hybrid gene comprising the coding sequences for a full length Smad and a DNA binding domain;
ii) a cDNA library, derived from a single tissue or cell type, cloned into a vector which fuses the library sequences to a transactivation domain; and
iii) a reporter gene, comprising a DNA sequence bound by said DNA binding domain, and transactivated by said transactivation domain;

20 b) selecting yeast cell clones which express said reporter gene;
c) probing DNA isolated from said clones with probes specific for all known Smad interactor proteins to identify clones which are novel; and
d) using the sequences of clones identified in c) as probes of multi-tissue Northern (RNA) blots to confirm tissue-specific expression of said clones identified in c).

25 39. A method to identify novel Smad proteins comprising the steps of:

- a) transforming yeast cells with expression constructs comprising:

- TELETYPE
- 5 i) a hybrid gene comprising the coding sequences for a full length Smad and a DNA binding domain;
- ii) a cDNA library, cloned into a vector which fuses the library sequences to a transactivation domain; and
- 10 5 iii) a reporter gene, comprising a DNA sequence bound by said DNA binding domain, and transactivated by said transactivation domain;
- b) selecting yeast cell clones which express said reporter gene;
- c) probing DNA isolated from said clones with nucleic acid probes derived from known Smads under conditions which permit the identification of yeast colonies which contain sequences which hybridize with known Smad sequences;
- d) isolating and sequencing the plasmid DNA sequences identified in c); and
- e) comparing the resulting sequences with known Smad sequences, such that clones with sequences which are not identical to the sequence of any known Smad are identified as novel.
- 15 40. A composition comprising a ternary complex comprising Smad1, HsN3 and antizyme.
41. A composition comprising a quarternary complex comprising Smad1, Smad4, HsN3 and antizyme.
42. A composition comprising one or more of antizyme and HsN3; ubiquitin and
20 HsN3; HEF1 and HsN3; or HEF1 and antizyme.

43. A composition comprising the SNIP1 and CBP/p300.